bP0 increased 10-fold by 48 hrs., gradually increasing to 100-fold by week 3 and then did not change significantly. In the co-cultures Sox9 expression increased 10-fold by 48 hrs. and remained stable up to 4 weeks.
Conclusions: Redifferentiation of passaged cells occurs within the first two weeks of co-culture with primary chondrocytes. The primary cells cultured alone, formed cartilage tissue one week earlier than the co-cultured cells. This difference may be due to the time needed for the passaged cells to undergo re-differentiation similar to that seen for mesenchymal stem cells undergoing differentiation to chondrocytes. Interestingly, lower levels of Sox9 gene expression in co-cultures was sufficient to result in Col2 expression similar to bP0. The data suggests that implantation of tissue must occur after 2 weeks of co-culture.

201

IMPLICATION OF INORGANIC PYROPHOSPHATE AND ITS TRANSPORTER ANK IN THE MAINTENANCE OF ARTICULAR CHONDROCYTE PHENOTYPE. ROLE OF WNT-5A

F. Cailotto, S. Sebillaud, D. Moulin, P. Netter, J.-Y. Jouzeau, A. Bianchi
PPIA UMR 7561 Nancy Université CNRS, Vandoeuvre les Nancy, France

Purpose: Articular chondrocyte phenotype is characterized by an expression pattern of genes coding for the extracellular matrix. Various wnt genes were described to induce a chondrocyte dedifferentiation process mediated by interleukin-1β (IL-1β) in osteoarthritis. Inorganic pyrophosphate (PPi) was shown to influence osteo-articular cells phenotype. Moreover, we previously demonstrated that ANK was mainly responsible for extracellular PPi (ePPi) generation. In the present work, we studied the role of ANK and ePPi in the maintenance of articular chondrocyte phenotype, known to be dedifferentiated in osteoarthritis. We focused on the implication of the Wnt signaling in this process.
Methods: We characterized the dedifferentiating effect of IL-1β (10 ng/ml) on the articular chondrocytes. To investigate the role of Ank in phenotype maintenance, we either transfected chondrocytes with a plasmid overexpressing Ank, or with siRNA directed against Ank. We analyzed genes expression by real-time quantitative RT-PCR, and performed immunocytochemestry analyses. We also harvested the supernatant from cells transfected with Ank siRNA, and used it on chondrocytes to check for the activation of canonical (Tcf/Lef reporter plasmids, western blot of β-catenin nuclear translocation) and non-canonical Wnt pathway (western blot of JNK phosphorylation). These experiments were repeated with neutralizing Wnt-5a antibodies to evaluate the contribution of Wnt-5a. In another set of experiment, we stimulated chondrocytes with supernatant from cells transfected with Ank siRNA and challenged with 0.1 mM of exogenous PPi. Type II collagen and Sox-9 expression were assessed, as well as the activation of Tcf/Lef. Lastly, we controlled whether the effect of IL-1β on the activation of JNK and on the expression of genes specific of chondrocyte phenotype were modulated by exogenous PPi.
Results: IL-1β induced chondrocyte dedifferentiation, as Wnt-5a mRNA expression was up-regulated by 2.5-fold, while Ank and type II collagen expression were reduced respectively by 3-fold and 4-fold. In cells overexpressing Ank, IL-1β induced no more Wnt-5a expression and type II collagen expression was only reduced for 55%. Transient Ank knock-down also led to dedifferentiation, as type II collagen and Sox-9 expression were reduced respectively by 50% and 35%, while Wnt-5a expression was induced 2.5-fold. Immunocytochemestry confirmed these tendencies. This suggested a role of Ank in articular chondrocyte phenotype maintenance. Supernatant from cells transfected with Ank siRNA induced a 2-fold activation of Tcf/Lef plasmid and an increase in nuclear β-catenin level, but no phosphorylation of JNK was detected. This showed the involvement of only Wnt canonical pathway in the process. Moreover, neutralization of supernatant using Wnt-5a antibody ensured a complete suppression of Tcf/Lef activation, demonstrating the crucial role of Wnt-5a in the dedifferentiation process. Exogenous PPi diminished the effect of Ank siRNA, as expression of type II collagen was less reduced (25% instead of 50%), and likely, Tcf/Lef activation was reduced by 50%. Finally, exogenous PPi did not modify JNK activation by IL-1β, but reduced its dedifferentiating effect, as type II collagen expression was only reduced by 2-fold (instead of 4-fold), suggesting that PPi only countered the canonical Wnt pathway-induced dedifferentiation process.
Conclusions: ANK and ePPi are implicated in articular chondrocyte phenotype maintenance, markedly resulting from suppression of Wnt canonical pathway activation. This opens new insights in the understanding of the Wnt signaling mechanisms in osteoarthritis.

202

DIFFERENTIAL ROLES OF THE IR AND THE IGFR IN MEDIATING INSULIN-INDUCED GLUCOSE TRANSPORT IN NORMAL AND OSTEOARTHRITIC HUMAN CHONDROCYTES

S.C. Rosa[1,2], F.M. Judas[3], M.C. Lopes[1,2], A.F. Mendes[1,2]
[1]Ctr. for NeuroSci. and Cell Biology, Coimbra, Portugal; [2]Faculty of Pharmacy, Univ. of Coimbra, Coimbra, Portugal; [3]Orthopedics and Bone Bank Dept., Univ. Hosp. of Coimbra, Coimbra, Portugal

Purpose: Some studies indicate that insulin can be an important anabolic factor for chondrocytes, namely due to its ability to promote proteoglycan and type II collagen synthesis. However, little attention has been devoted to the mechanisms that mediate the actions of insulin in chondrocytes, particularly whether the specific insulin receptor (IR) or the structural- and functionally-related insulin-like growth factor receptor (IGFR) is involved. Glucose is essential for glycosaminoglycan synthesis and one of the major actions of insulin is to increase glucose transport, namely in some cells by promoting the plasma membrane incorporation of the Glucose Transporter (GLUT)-1, which is expressed by chondrocytes and regulated by anabolic and catabolic stimuli. Thus, the aim of this study was to determine whether 1) adult human chondrocytes express the IR, 2) physiological concentrations of insulin are able to regulate glucose transport and GLUT-1 protein content in normal and osteoarthritic (OA) human chondrocytes, and 3) the effects of insulin on glucose transport are mediated by the IR or the IGFR.
Methods: Normal human chondrocytes were isolated from the femoral condyles of multi-organ donors without macroscopic signs of arthritic lesions. OA chondrocytes were isolated from patients undergoing total knee replacement surgery. Serum-starved subconfluent chondrocyte cultures were treated with 1 or 10 nM insulin for 48h or 30 minutes in the absence or presence of 0,05 or 0,5 μM Picropodophyllotoxin (PPP), an IGFR kinase inhibitor. Glucose uptake was measured as the amount of non-metabolizable 2-Deoxy-D-2-[2,6-^{3}H]glucose (2-DG uptake) transported into chondrocytes for 30 minutes at 37°C. Phosphorylation of Akt and total GLUT-1 content were evaluated by Western blot in whole cell lysates. IR mRNA expression was evaluated in normal and OA chondrocytes by Real time RT-PCR.
Results: Similar levels of IR mRNA were detected in both normal and OA chondrocytes. Treatment with 1 or 10 nM insulin for 48h significantly increased 2-DG uptake (125±3.6% and 141±3.5%, respectively) in normal chondrocytes. The presence of PPP caused an 8 and 20% reduction in 2-DG uptake induced by